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[^{3}H]DYNORPHIN A BINDING AND κ SELECTIVITY OF PRODYNORPHIN PEPTIDES IN RAT, GUINEA-PIG AND MONKEY BRAIN

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We have previously demonstrated that [^{3}H]dynorphin A selectively labels κ opioid receptors in guinea-pig whole brain. In these current studies, using protection from inactivation by β-chloronaltrexamine (β-CNA), we are able to demonstrate that although dynorphin A prefers κ receptors, it will label μ receptors when κ receptors are not available, or present in only a small number. Thus, differences in numbers of μ and κ receptors present in brain preparations are critical in determining the receptor binding profile of [^{3}H]dynorphin A across species. Additionally, although all the prodynorphin derived peptides show κ preference, the ability of the other prodynorphin derived peptides to compete with [^{3}H]dynorphin A for its receptor varies across species. Consequently, in a highly enriched κ preparation such as monkey cerebral cortex, [^{3}H]dynorphin A appears to label κ receptors with substantial selectivity, and the other prodynorphin-derived peptides show less ability to compete with dynorphin A for its receptor. In contrast, in a κ-poor tissue such as rat brain, all of the prodynorphin-derived peptides, including dynorphin A-(1-8), show very similar potency. Thus, differences in μ and κ receptor numbers across brain regions and species lead to differences in the receptor binding profile of dynorphin A.

Multiple opiate receptors Dynorphin

1. Introduction

The relationship between multiple forms of endogenous opioid peptides and various subtypes of opioid receptors is still being elucidated. The discovery of dynorphin (Goldstein et al., 1979), the elucidation of its precursor (Kakidani et al., 1982), and the realization that multiple active opioids are derived from it (Weber et al., 1982; Suda et al., 1982; Kilpatrick et al., 1982; Fischli et al., 1982) have led to the attempt to define the receptor profiles of the prodynorphin products (Chavkin et al., 1982; Corbett et al., 1982; James et al., 1982).

Using guinea-pig ileum, James et al. (1984) have characterized the pharmacological selectivity of the prodynorphin products for the κ receptor in that tissue. Although dynorphin A is the most potent of these peptides, dynorphin A, α-neo-endorphin and dynorphin B all are highly κ selective. In contrast, dynorphin A-(1-8), α-neo-endorphin, and dynorphin B 29 appear to be less κ selective. Corbett et al. (1982), using guinea-pig whole brain, have shown that dynorphin A has the highest affinity at the κ site, while dynorphin A-(1-9) and α-neo-endorphin show only slightly lower affinity than dynorphin A. In contrast, dynorphin A-(1-8) shows ten-fold less affinity for the κ site than dynorphin A, although it still prefers κ to μ or δ sites. However, not all reports agree as to the

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degree of selectivity for the κ receptor exhibited by dynorphin A, dynorphin B, and α-neo-endorphin in brain preparations. While several reports demonstrate such a selectivity in the guinea-pig brain (Chavkin et al., 1982; Corbett et al., 1982; James et al., 1984), other reports suggest significant interactions of dynorphin with the μ receptor (Quirion and Pert, 1981; Hewlett and Barchas, 1984). Since a number of these studies employed competition by the peptides against labeled 'μ' and 'κ' alkaloids, it is possible that part of the confusion derived from the selectivity of the labeling ligands and the conditions of the assay. Therefore, we attempted to use [^{3}H]dynorphin A to label and characterize brain opioid binding sites (Young et al., 1983a; Lewis et al., 1984).

Based on our work in the guinea-pig brain homogenates, we concluded that [^{3}H]dynorphin A labels promarily κ sites as suggested by others (Corbett et al., 1982; Wüster et al., 1980; 1981; Chavkin et al., 1982; James et al., 1984). However, in a preliminary report (Young et al., 1983b), we noted differences in the receptor selectivity of [^{3}H]dynorphin A in guinea-pig and rat brain. While the binding of [^{3}H]dynorphin A appeared to be extremely κ selective in guinea-pig it was less so in rat brain. These differences paralleled those noted across various studies when other κ ligands, e.g. MR 2034 (UM 1071) and ethylketocyclazocine (EKC), were used (Hiller and Simon, 1980; Pfeiffer and Herz, 1982; Gillan and Kosterlitz, 1982). This is probably due to a difference in the number of κ sites in the two species with guinea-pig having approximately 2-3 times as many κ sites as rat brain, while μ sites are more numerous in rat brain (Gillan and Kosterlitz, 1982). Thus, some of the confusion regarding κ selectivity of putative κ ligands may result from the use of rat vs. guinea-pig to characterize κ binding. That is, a ligand that is κ selective in guinea-pig brain shows less apparent κ selectivity in κ-poor tissues such as rat brain. In this paper, we address the issue directly.

Using both [^{3}H]dynorphin A and [^{3}H]UM 1071 (the active stereoisomer of MR 2034, a putative κ ligand) we examined the relative κ selectivity of the prodynorphin peptides in rat, guinea-pig and monkey brain. Since dynorphin A may label sites other than κ sites, particularly in a κ-poor tissue, we evaluated, the ability of the prodynorphin peptides to compete with [^{3}H]dynorphin A for its sites. We were particularly interested in monkey brain since prototypical κ ligand appear to be highly selective for κ receptors in vivo in monkey (Hein et al., 1981). Using guinea-pig brain and rat brain homogenates as the prototypical κ-rich and κ-poor tissues respectively, we compared [^{3}H]dynorphin A binding in monkey cerebral cortex to that in guinea-pig and rat brain. To characterize the receptor profile of dynorphin A binding, we used both competition of various ligands against [^{3}H]dynorphin A and [^{3}H]UM 1071 binding, and selective protection from β-CNA inactivation by μ and κ ligands. While we observe substantial κ preference of the prodynorphin peptides in brain, we also report significant species differences in the receptor binding profile of [^{3}H]dynorphin A as well as in the ability of prodynorphin peptides to compete with dynorphin A for its receptor.

2. Materials and methods

Receptor binding assays for [^{3}H]UM 1071 were performed as described by Young et al. (1983c). [^{3}H]Bremazocine binding assays in the presence of 100 nM [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAGO) and 100 nM [D-Ser^2,Leu^5]enkephalin-Thr (DSLET) were performed at 4°C in a similar fashion. Rat and guinea-pig brain minus cerebellum were homogenized in 50 mM Tris buffer (pH 7.4 at 25°C) with 5% DMSO added. Several rat or guinea-pig whole brains were pooled for homogenization to assure differences between tissue preparations were attributable to between species rather than between animal variation. Rhesus monkey cortex was frozen on dry ice and stored at −70°C until ready for use. Random pieces of cortex, 1 g in weight, were used for each binding assay. Previous studies demonstrated no changes between fresh and frozen tissue in μ, δ or κ receptor binding using [^{3}H]morphine, [^{3}H][D-Ala^2,D-Leu^5]enkephalin (DADL), [^{3}H]UM 1071 or [^{3}H]dynorphin A as labeling ligands (Young et al., 1983c).

[^{3}H]Dynorphin A binding was performed as described previously (Young et al., 1983a). In brief,

[^{3}H]dynorphin A binding was carried out in 50 mM Tris buffer. Dynorphin A, α-neo-endorphin, dynorphin B and dynorphin A (1-8) were added in 10 μl volume in 1 : 1 mixture of methanol and 0.1 N HCl. [^{3}H]Dynorphin A was added in 100 μl volume to yield a final concentration in the assay of 0.5-1 nM. Fifteen milligrams (wet weight) of crude membrane fragments were added to each tube in a total volume of 390 μl. After 90 min of incubation at 4°C, the bound [^{3}H]dynorphin A was separated from the free by rapid filtration under vacuum over Whatman GF/B glass fiber filters which had been presoaked in 50 mM Tris buffer containing 0.4% BSA (Fraction V, Sigma) and 0.1% polylysine (Sigma). Tris buffer with 0.1% BSA, 100 mM choline chloride and 0.01% Triton X-100 ([^{3}H]β-endorphin washing buffer (Law et al., 1979)) was used as the washing buffer. Using this procedure, [^{3}H]dynorphin A binding to filters is reduced to approximately 5%. UM 1071 (a putative κ ligand) or levorphanol, 1 μM, was used to define non-specific binding. Specific binding of [^{3}H]dynorphin A to guinea-pig and monkey brain averaged 50%. Specific binding to rat brain, under the same conditions, averaged 40%.

Protection from irreversible binding to the alkylating agent β-chloronaltrexamine (β-CNA) by either morphine or dynorphin was performed as described by James et al. (1982) with the following modifications: protection and β-CNA inactivation was performed in 50 mM Tris buffer rather than Krebs buffer, and these reactions are carried out with brain tissue at a concentration of 50 mg/ml. The concentration of dynorphin A used for protection was 10 nM and 100 nM. The concentration used for morphine protection was 10 nM, since morphine shows the greatest μ selectivity in the range of 10 nM or less.

After incubation at 37°C for 20 min to dissociate endogenous ligands, the membranes were centrifuged at 40 000 × *g* for 10 min, resuspended in 50 mM Tris buffer at a concentration of 50 mg tissue/ml buffer, then incubated 5 min at 37°C with the protecting ligand. β-CNA was added at concentrations of 100 nM to 1 μM for 15 min at 37°C to inactivate other opiate receptors. After 15 min, the membranes were diluted four-fold in Tris buffer than centrifuged at 25 000 × *g* for 5 min, resuspended in 50 mM Tris buffer then centrifuged again for a total of six washes. After these washes, the membranes were incubated at 37°C for 20 min, then washed two more times before resuspension in Tris buffer at the final concentration of 37.5 mg tissue/ml buffer for the binding assay. All binding kinetics were done with iterative non-linear computer analysis (Ligand, Munson and Rodbard). Curves for IC_{50}s were drawn by hand, but represented a mean of at least three determinations.

3. Results

3.1. Dynorphin A interaction with μ and κ sites in rat and guinea-pig

Binding data using [^{3}H]UM 1071 as the labeling ligand for κ sites showed the same K_D for rat and guinea-pig (rat = 1.4 ± 0.6; guinea-pig = 1.3 ± 0.5). In contrast the B_{max} was two-fold different between species (rat B_{max} = 227 ± 18 pM; guinea-pig B_{max} = 423 ± 52 pM). Using [^{3}H]bremazocine in the presence of 100 nM DAGO and DSLET to define κ binding we observe similar K_D of bremazocine at the κ site between rat and guinea-pig (rat = 0.15 ± 0.2; guinea-pig = 0.28 ± 0.10). The K_I for dynorphin A at the κ site is not significantly different between these two species (rat K_I dynorphin A = 0.2 ± 0.1; guinea-pig K_I dynorphin A = 0.34 ± 0.13). Likewise using [^{3}H]DAGO to define μ sites, the K_I of dynorphin A at the μ site does not differ between rat and guinea-pig (rat = 1.3 ± 0.1, guinea-pig = 1.4 ± 0.2). Thus, apparent differences in [^{3}H]dynorphin A binding between these species cannot be attributed to different affinities at the μ and κ site, but rather differences in the proportion of μ and κ sites labeled by [^{3}H]dynorphin A between these species.

3.2. [^{3}H]Dynorphin A binding in rat and guinea-pig

Previous experiments using [^{3}H]dynorphin A in rat and guinea-pig brain suggested substantial differences between species in the ability of morphine to displace [^{3}H]dynorphin A binding (Young et al., 1983b). In rat, the IC_{50} of morphine in compe-

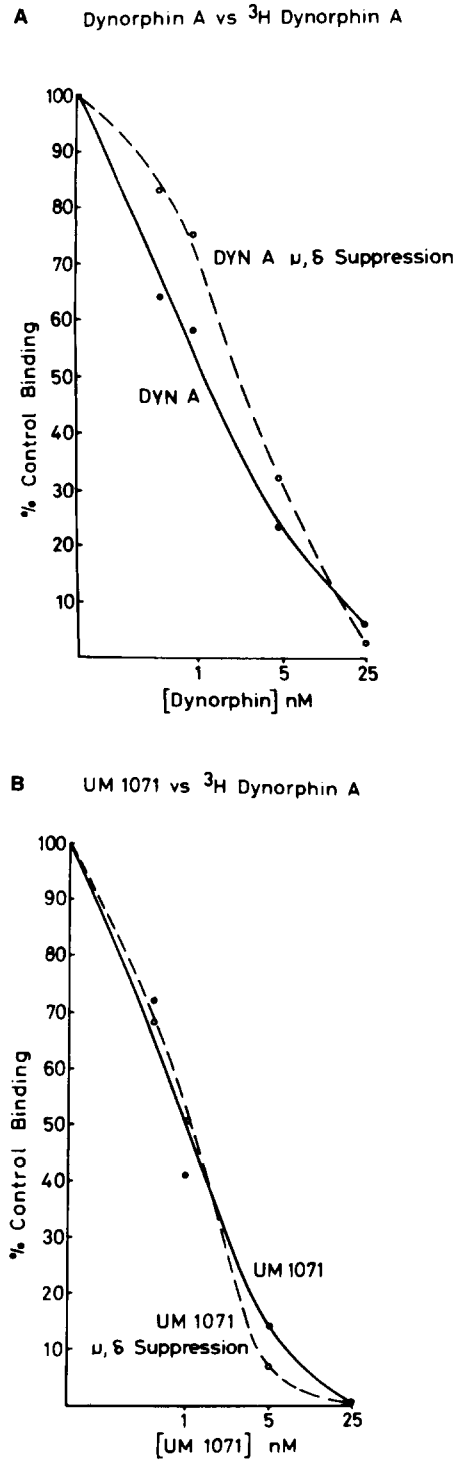

Fig. 1. The competition curves of dynorphin A and UM 1071 against [^{3}H]dynorphin A in the absence and presence of μ and δ selective ligands at blocking concentrations in guinea-pig whole brain homogenates. Addition of 100 nM morphine and DADL resulted in only slight shift in the IC_{50} of dynorphin A (A) and no shift in the IC_{50} of UM 1071 (B).

tition with [^{3}H]dynorphin A is substantially lower than the IC_{50} of morphine versus [^{3}H]dynorphin A in guinea-pig brain, suggesting that [^{3}H]dynorphin A labels both μ and κ receptors in rat brain. To further characterize the μ and κ nature of [^{3}H]dynorphin A binding, experiments using suppression of μ and δ binding were undertaken in rat and guinea-pig brain. Addition of 100 nM morphine plus 100 nM DADL to [^{3}H]dynorphin A binding in rat brain results in the loss of 90% of the binding, again suggesting that [^{3}H]dynorphin A labels both μ and κ receptors in rat brain. In guinea-pig brain (fig. 1A) there is a slight shift in the IC_{50} of dynorphin A against itself (1.2-2.4 nM) in the presence of morphine and DADL. In contrast, the IC_{50} of UM 1071 against [^{3}H]dynorphin A binding is unchanged by the addition of 100 nM morphine and DADL (IC_{50} = 1 nM in both cases) (fig. 1B).

Further support for the labeling of a mixture of μ and κ sites in brain can be demonstrated by equilibrium binding kinetics. Such studies must be interpreted with caution, since breakdown occurs during the course of the incubation, and the addition of unlabeled dynorphin can change the rate of breakdown of the labeled ligand. Consequently, effort was made to include only those concentration ranges over which breakdown is constant for these computations. The apparent K_D of dynorphin A in rat brain is higher than the apparent K_D in guinea-pig brain (rat = 2.9 $\pm$ 0.7, guinea-pig = 1.3 $\pm$ 0.25). The number of sites labeled in both species is similar (rat = 226 $\pm$ 43, guinea-pig = 174 $\pm$ 22). This is not surprising, since dynorphin A is an endogenous ligand, and should have ample receptors in both species. The higher K_D in rat brain would be expected if [^{3}H]dynorphin A is also labeling μ receptors, towards which dynorphin A has a lower affinity than it has for κ receptors.

TABLE 1

β-CNA inactivation and protection (% untreated tissue binding).

	Control (untreated tissue)	β-CNA treatment			Washing controls	
		CNA alone	10 nM Dyn + CNA	10 nM Mor + CNA	10 nM Dyn A alone	10 nM Mor alone
[^{3}H]Dihydromorphine (0.5 nM)	100	11	0	84	100	84
[^{3}H]Dynorphin A (0.5 nM)	100	34	89	66	98	84

3.3. β-CNA inactivation studies with [^{3}H]dynorphin A

Since [^{3}H]dynorphin A appears to label both μ and κ receptors in rat brain, β-CNA inactivation and protection experiments to demonstrate the κ selectivity of [^{3}H]dynorphin A binding were undertaken only in guinea-pig brain, a κ-rich tissue. The protection from β-CNA inactivation experiments (table 1) again shows that 10 nM dynorphin A can protect [^{3}H]dynorophin A binding sites with approximately 90% recovery of the sites. The non-μ nature of these sites is indicated by the failure of [^{3}H]dihydromorphine to label these sites. Higher protecting concentrations (100 nM) of dynorphin A resulted in a low recovery of [^{3}H]dynorphin A binding after washing. While morphine (10 nM) protects [^{3}H]dihydromorphine sites from inactivation by β-CNA morphine (tables 1 and 2), these morphine or μ sites can also be labeled by [^{3}H]dynorphin A. The μ nature of this [^{3}H]dynorphin A binding is demonstrated by a six-fold decrease in the IC_{50} of morphine against [^{3}H]dynorphin A (table 2). In contrast, there is no shift in the IC_{50} of morphine against [^{3}H]dihydromorphine. This suggests that although dynorphin A prefers κ sites, it will label μ sites when κ sites are no longer available. This is consistent with the data on [^{3}H]dynorphin A binding in rat brain, a κ-poor tissue, which shows that [^{3}H]dynorphin A labels a substantial number of μ as well as κ receptors in rat brain. Of interest is the finding that [^{3}H]dynorphin A binding sites appear to be more resistant to inactivation by β-CNA than [^{3}H]dihydromorphine sites, so that concentrations of 100 nM to 1 μM β-CNA, which appear to inactivate 90% of dihydromorphine sites, show between 50-70% inactivation of [^{3}H]dynorphin A sites.

3.4. Studies in the rhesus monkey

Since [^{3}H]dynorphin A appears to label primarily κ sites in guinea-pig brain, but both μ and κ sites in rat brain, the question of tissue-dependent ligand selectivity was pursued in another species, rhesus monkey, in which κ receptors have been explored at a behavioral level (Hein et al., 1981). Again, substantial species differences in [^{3}H]dynorphin A binding to μ and δ receptors are seen (figs. 2A, 3C, 4E and table 3). Similar species differences were observed in competition studies using [^{3}H]UM 1071 as a putative κ ligand (figs. 2B, 3D, 4F). These studies indicate that, with respect to κ selectivity, it appears that monkey cerebral cortex more closely resembles guinea-pig

TABLE 2

Selectivity of morphine protected [^{3}H]dynorphin A binding sites after β-CNA inactivation.

Conditions	Control	β-CNA + 10 nM Mor	β-CNA alone
[^{3}H]Dihydromorphine			
Remaining binding (%)	100	84	11
Morphine IC_{50} (nM)	2	2	Insufficient binding
[^{3}H]Dynorphin A binding			
Remaining binding	100	66	34
Morphine IC_{50}	75 nM	13 nM	300nM

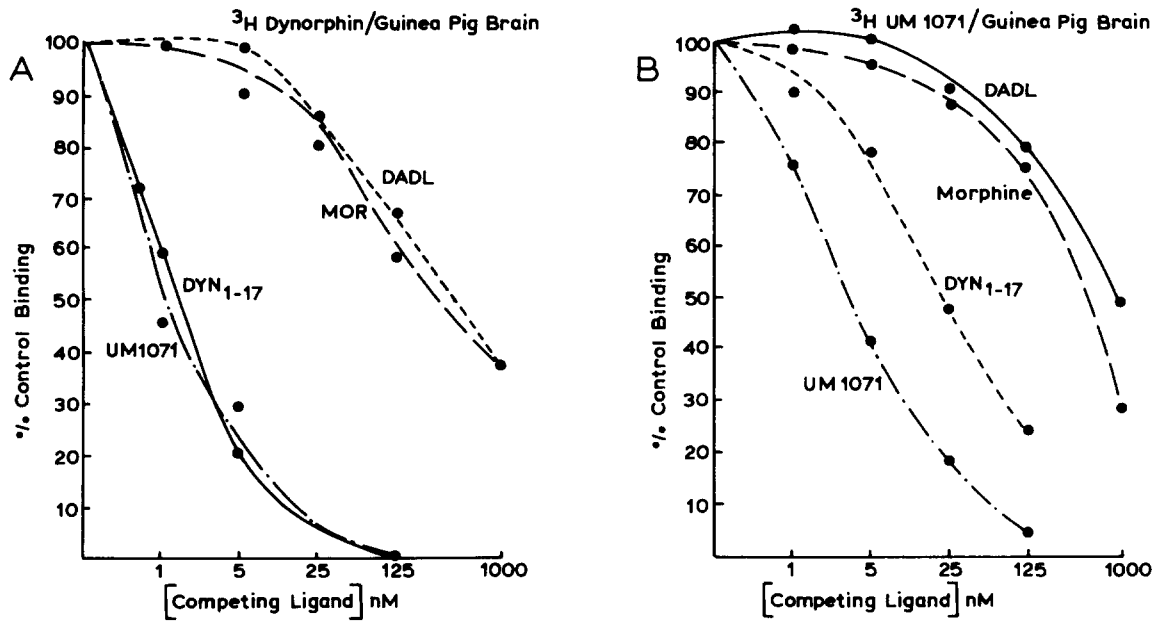

Fig. 2. The competition curves of dynorphin A, UM 1071, morphine and DADL against [^{3}H]dynorphin A and [^{3}H]UM 1071, a κ selective opiate alkaloid in guinea-pig brain. Both [^{3}H]dynoprhin A and [^{3}H]UM 1071 label a site towards which morphine and DADL show only very low affinity.

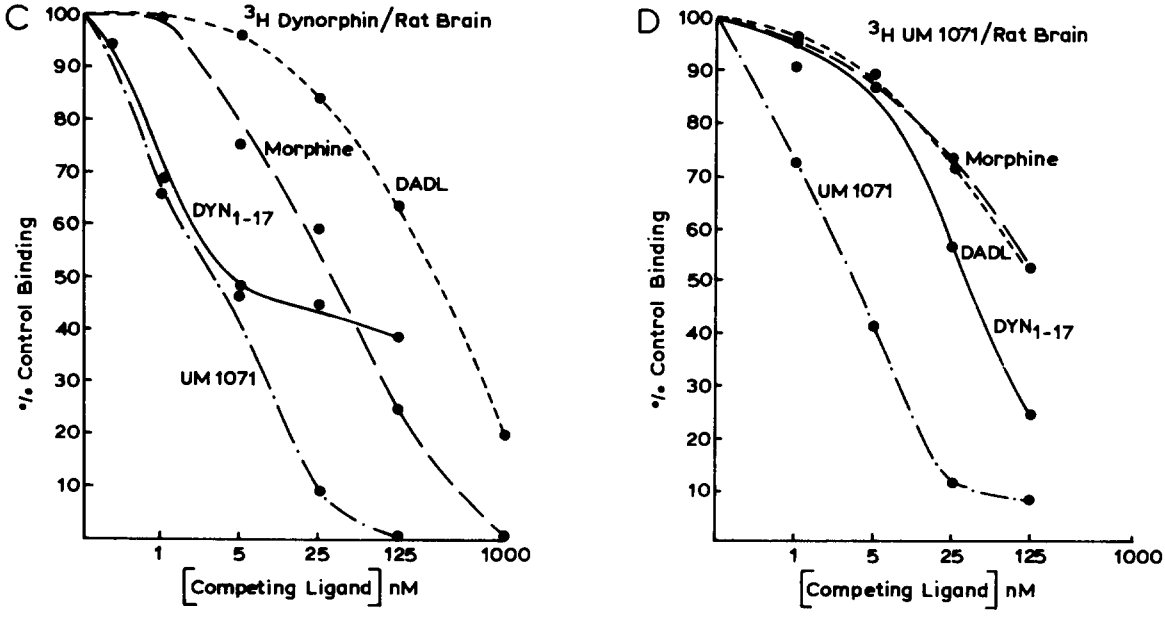

Fig. 3. The competition curves of dynorphin A, UM 1071, morphine and DADL against [^{3}H]dynorphin A and [^{3}H]UM 1071 in rat brain. In rat brain, morphine shows a 20-fold lower IC_{50} against [^{3}H]dynorphin A binding than in guinea-pig brain, indicating that [^{3}H]dynorphin A is less κ selective in rat brain. The IC_{50} of morphine against [^{3}H]UM 1071 binding is only 3-fold lower in rat brain than guinea-pig brain.

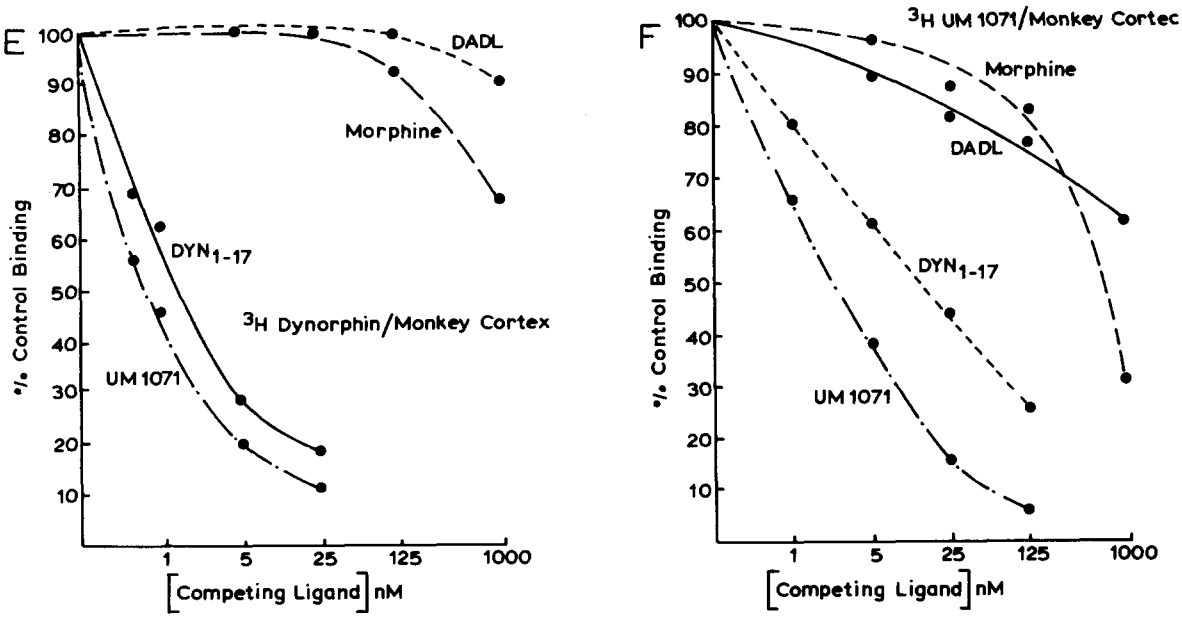

Fig. 4. The competition curves of dynorphin A, UM 1071, morphine and DADL against [^{3}H]dynorphin A and [^{3}H]UM 1071 in monkey cortex. In monkey cerebral cortex both [^{3}H]dynoprhin A and [^{3}H]UM 1071 appear to label a similar site that is very non-μ and non-δ, suggesting a profile similar to guinea-pig whole brain.

TABLE 3

IC_{50} of various opiate ligands vs. [^{3}H]dynorphin A (nM).

	Rat	Guinea-pig	Monkey
Morphine	32	680	>1000
DADL	300	>1000	>1000
Dynorphin A	2.4	1.2	0.8
UM 1071	2.0	1.1	0.7

than rat brain. Both [^{3}H]dynorphin A and [^{3}H]UM 1071 appear to label κ sites more exclusively in monkey cortex than guinea-pig whole brain (fig. 3). Both dynorphin A and UM 1071, show a 500-1000-fold lower IC_{50} than morphine or DADL against [^{3}H]dynorphin A or [^{3}H]UM 1071 binding in guinea-pig or monkey brain (figs. 2, 3, 4 and table 3).

3.5. *Displacement profile of pro-dynorphin products*

Comparing the prodynorphin peptides among three species, the similarity of dynorphin A, dynorphin B and α-neo-endorphin in displacing [^{3}H]dynorphin A is quite evident (table 4). However, dynorphin A-(1-8) has more difficulty in competing with [^{3}H]dynorphin A in all species. This is demonstrated in guinea-pig brain, where

TABLE 4

IC_{50} of prodynorphin peptides vs. [^{3}H]dynoprhin A (1 nM).

	DYN A	DYN B	DYN A-(1-8)	α-Neo-End	$\frac{IC_{50}\ DYN\ A\text{-}(1\text{-}8)}{IC_{50}\ DYN\ A}$
Rat	4.5 nM ± 0.6	4.0 nM ± 1.3	11 nM ± 5	1.6 nM ± 0.1	2.4
Guinea-pig	1.2 nM ± 0.3	1.8 nM ± 0.5	14 nM ± 0.7	2.0 nM ± 1	12
Monkey	0.7 nM ± 0.2	2.6 nM ± 1.6	9.5 nM ± 0.6	2.4 nM ± 1.4	(13.6)

the ratio of IC_{50} dynorphin A-(1-8) to the IC_{50} of dynorphin A is 12, in contrast to rat brain where the ratio is 2.4. Using [^{3}H]UM 1071 as a labeling κ ligand, a very similar pattern is seen, where dynorphin A, dynorphin B and α-neo-endorphin appear equivalent but dynorphin A(1-8) shows a four-fold higher IC_{50} than dynorphin A. Previous work by Corbett et al. (1982) in guinea-pig brain has shown that dynorphin A-(1-8), while demonstrating κ affinity retains μ and δ receptor properties. Thus, the processing of dynorphin A to dynorphin A-(1-8) differentially affects its κ selectivity among species.

4. Discussion

The present studies provide evidence that prodynorphin-derived peptides can exhibit marked selectivity for κ binding sites. Our data are consistent with previous experiments by James et al. (1984) with guinea-pig ileum showing that dynorphin A, dynorphin B and α-neo-endorphin show similar pharmacological κ selectivity, while dynorphin A-(1-8) shows less κ selectivity than these other prodynorphin peptides. Similarly, Corbett et al. (1982) using [^{3}H]bremazocine in the presence of unlabeled μ and δ ligands, have shown dynorphin A, dynorphin A-(1-8), dynorphin A-(1-9), and α-neo-endorphin are selective for κ receptors in guinea-pig brain. However, our results indicate dynorphin A sites are not necessarily identical to the κ site. Thus, dynorphin A binding may represent a preponderance of κ binding along with a proportion of μ binding depending on the species and possibly brain region under study. For example, in the β-CNA selective protection studies with guinea-pig brain, sites protected with dynorphin A later showed binding of [^{3}H]dynorphin A, but not [^{3}H]dihydromorphine, a finding consistent with the κ selectivity hypothesis. However, in contradiction to this hypothesis, μ sites protected with morphine also showed binding of [^{3}H]dynorphin A, with increased sensitivity to displacement by morphine. As discussed below, the κ selectivity issue was also addressed in experiments which exploited species differences in the relative abundance of opioid receptor subtypes.

When comparing across the three species and tissue preparations, rat brain, guinea-pig brain and monkey cortex, the κ selectivity of dynorphin A becomes increasingly evident. In rat, dynorphin shows a low κ selectivity in contrast to guinea-pig whole brain or monkey cortex. This increasing κ selectivity is reflected in the progressive decrease in the IC_{50} of dynorphin A against itself, and the progressive increase in the IC_{50} of morphine and DADL against [^{3}H]dynorphin A. This same cross-species pattern is obtained using [^{3}H]UM 1071, a κ opiate alkaloid, indicating that monkey cerebral cortex is highly enriched in κ opioid receptors. This agrees with other work showing κ receptors present in the deep cortex of guinea-pig (Goodman and Snyder, 1982), as well as our own work showing that [^{3}H]dynorphin A labels these same deep cortical layers in guinea-pig brain as does [^{3}H]bremazocine in the presence of unlabeled μ and δ ligands (Lewis et al., 1984; Lewis et al., unpublished observations). This also supports much of the in vivo behavioral data with κ compounds in primates (Hein et al., 1982).

By using [^{3}H]dynorphin A as the labeling ligand, we are able to compare directly the ability of these prodynorphin peptides to compete for the dynorphin A binding site across the species. Thus, in guinea-pig, dynorphin A, dynorphin B and α-neo-endorphin appear to compete well for this same 'dynorphin A' site, while dynorphin A-(1-8) shows less ability to compete with dynorphin A, its parent/precursor peptide. In contrast, in rat brain, the ratio of the IC_{50} of dynorphin A to the IC_{50} of dynorphin A(1-8) is much lower, suggesting that the processing of dynorphin A to dynorphin A-(1-8) has less impact on its ability to compete with dynorphin A for the same receptor. This species difference in the ability of dynorphin A-(1-8) to compete for dynorphin A receptors may be dependent upon the difference in numbers μ and κ receptors in these systems. Thus, in situations where dynorphin A labels μ as well as κ receptors, dynorphin A-(1-8) is able to compete well for dynorphin A binding sites. This suggests that, in rat, the processing of dynorphin A to dynorphin A-(1-8) results in less change in receptor selectivity of the product dynorphin A-(1-8) than pharmaco-

logical studies in guinea-pig may suggest. This is not the case for guinea-pig or monkey brain where dynorphin A-(1-8) is much less able to compete with dynorphin A than in rat. It is of interest that work by Weber et al. (1982), Dores et al. (1985) and Lewis et al. (1985) have shown a predominance of dynorphin A-(1-8) in most brain regions of rat, while in guinea-pig there is substantially less processing of dynorphin A to dynorphin A(1-8).

Recent work by Quirion and Pilapil (1984) with [^{3}H]dynorphin A(1-8) in rat and guinea-pig brain suggest κ selectivity of this ligand. Binding and competition studies were conducted in the presence of 100 mM NaCl, which greatly reduces the ability of morphine to bind and compete with this ligand. Nonetheless, their data shows a five-fold lower IC_{50} of morphine in rat brain than guinea-pig brain, suggesting this ligand may label some μ receptors in rat brain. Likewise, Gillan et al. (1985) have also shown κ selectivity for [^{3}H]dynorphin A-(1-8). This supports earlier work that dynorphin A-(1-8) prefers κ although it is not as selective as dynorphin A, its parent compound (Corbett et al., 1982).

In monkey cerebral cortex, differences between the various prodynorphin peptides begin to emerge. Thus, dynorphin B and α-neo-endorphin appear to be less able to compete for the same receptors as dynorphin A in this tissue, since the IC_{50} of these peptides against [^{3}H]dynorphin A is approximately three-fold higher than the IC_{50} of dynorphin A against itself. While we did not see differences between dynorphin A and α-neo-endorphin in guinea-pig brain, the work in monkey cortex is in agreement with the work of Corbett et al. (1982) in guinea-pig brain showing that the K_i of α-neo-endorphin is two-fold higher than dynorphin A for the κ site. Thus, in a κ-enriched tissue such as cerebral cortex, dynorphin A more exclusively labels κ sites, and the IC_{50} of dynorphin A against itself decreases.

We cannot eliminate the possibility that, across species, differential breakdown of these prodynorphin peptides is responsible for the changes in their ability to compete with dynorphin A for its receptor. Indeed, in preliminary studies in guinea-pig brain, we have found that the presence of 1 μM α-neo-endorphin does not affect the amino peptidase action against [^{3}H]dynorphin A, while 1 μM dynorphin A substantially inhibits the breakdown of [^{3}H]dynoprhin A. This might suggest that different enzymes are responsible for the N terminal cleavage of tyrosine from these various opioid peptides. Such differences in breakdown of various prodynorphin peptides in vitro may be physiologically relevant, since, in vivo, these peptides are likely to be released simultaneously from the same terminals and would be competing for the same receptors. Consequently, differences in the number of opioid receptor types as well as differences in the receptor selectivity, processing to smaller forms, and breakdown of these prodynorphin peptides after release may all be cellular strategies for encoding biological information in the different prodynorphin peptides. The demonstration of a number on nonopioid effects produced by dynorphin A and dynorphin A-(2-17) (Walker et al., 1982a,b; Przewlocki et al., 1983) further supports the concept of multiple cores of information, both opioid and nonopioid, contained in the same prohormone. These differences in binding paradigms seen across species may product distinct behavioral and pharmacological profiles of these peptides in these different species.

In conclusion, [^{3}H]dynorphin A appears to selectively label κ receptors in κ receptor-rich tissues (guinea-pig brain and monkey cerebral cortex). Dynorphin B and α-neo-endorphin appear to compete well with dynorphin A for these sites in guinea-pig brain but somewhat less well in monkey cerebral cortex. The processing of dynorphin A to dynorphin A-(1-8) affects its ability to compete with dynorphin A for the same receptors in guinea-pig brain and monkey cerebral cortex. In contrast, this processing of dynorphin A to dynorphin A-(1-8) has less effect in rat brain, where dynoprhin A labels a less pure receptor population. There may be differences in the opioid inactivation of these prodynorphin peptides that further modify the actions of these peptides on receptors after their simultaneous release from nerve terminals. Such differences across species may result in different behavioral and pharmacological profiles for these prodynorphin peptides.

Even though dynorphin A is a highly selective κ

ligand and has a lower affinity to the μ sites, its high potency imparts to it a substantial ability to interact at the μ site. It appears that the other prodynorphin κ selective products (dynorphin B, α-neo-endorphin) possess similar characteristics. Thus, whether prodynorphin products act at μ or κ sites will in part depend on the relative abundance of receptors available in the vicinity of the prodynorphin terminals, which will vary as a function of brain region and species.

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